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Bioprinting Skin Tissue Model Protocol

GelXA SKIN

This is a suggested procedure, please adjust according to your experimental needs. To maintain the sterility of the product, work under sterile conditions.

Protocol aim

The aim of this protocol is to provide instructions for printing a skin tissue model using GelXA SKIN bioink, primary human fibroblast cells and primary human keratinocyte cells. The model has a fibroblast gradient mimicking papillary and reticular dermal compartments, with a higher fibroblast concentration in the papillary layer, see Figure 1. For a general protocol of bioprinting GelXA SKIN see *Bioprinting Protocol GelXA SKIN*.

Material needed

- CAD software
- Slic3r software
- USB Flash Drive
- Sterile conical bioprinting nozzles, 22G*
- 3 cartridges, 3cc*
- BIO X*
- (3 Temperature-controlled printheads*)
- GelXA SKIN*
- Primary human fibroblasts, HDF
- Primary human keratinocytes, HEK
- Cell culture medium
- Syringes and female/female Luer lock adaptor
- Empty, amber cartridges with end and tip cap, 3cc*
- (CELLMIXER*)
- 24-well plate
- Transwell inserts
- Crosslinking Agent (included with the bioink purchase)
- Thrombin, 100 U (included with the bioink purchase)

*The product can be purchased in the CELLINK shop at *www.cellink.com/store/*.

KEEP THE BIOINK PROTECTED FROM LIGHT IF TRANSFERRED FROM THE ORANGE UV PROTECTED CARTRIDGES TO AVOID CROSSLINKING BEFORE PRINTING. WORK WITH 3D PRINTERS IN DARK MODE. THE PHOTOINITIATOR IS SENSITIVE TO REPEATED OR PROLONGED EXPOSURE TO HEAT.

Protocol

This protocol can be performed with standard pneumatic printheads at room temperature and print bed cooled to 14°C, where room temperature is between 20-25°C. It is however recommended to use the Temperature-controlled printheads for best shape fidelity.

Step	Title	Material	Description
1	Creation of stl files needed for the model	- CAD software	Open the CAD software and create following 3 stl files, each representing a layer of the blueprint in Figure 1: - Top part (reticular dermis): • Shape: Rectangular • Size = 8 x 8 x 0.8 mm (2 layers of 0.4 mm) - Middle part (papillary dermis) • Shape: Rectangular • Size = 8 x 8 x 0.4 mm (1 layers of 0.4 mm) - Bottom part (epidermis): • Size = 8 x 8 x 0.4 mm • Layer height = 0.4 mm (1 layers of 0.4 mm) Save the parts as three separate stl files. Note: The model will be printed upside down and then flipped after crosslinking to maintain grid structure. Thereof the inverted order of the objects.

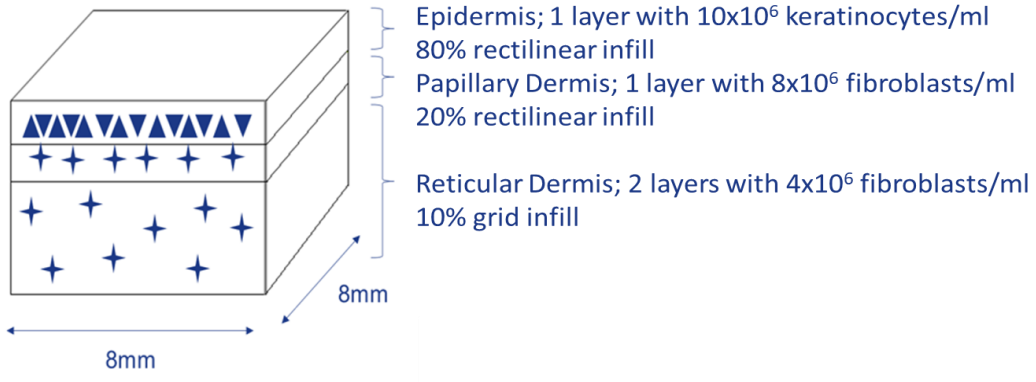

Figure 1. Blueprint of skin model.

2	Creation of G-code	- Slic3r - USB	- Open Slic3r. - Add the bottom part into Slic3r and open the object settings for the bottom part. In the object settings add the middle and top part in named order. This is to have all three stl objects in the same model. Opening all three files directly will generate three different models. - Make sure the parts are aligned and on top of each other and adjust the slicing parameters to following: - Bottom part (epidermis): • Printhead = 1 • Infill pattern = rectilinear • Infill density = 80% • Printing speed, F = 10 mm/s - Middle part (papillary dermis): • Printhead = 2 • Infill pattern = rectilinear • Infill density = 20% • Printing speed, F = 10 mm/s - Top part (reticular dermis): • Printhead = 3 • Infill pattern = grid • Infill density = 10% • Printing speed, F = 10 mm/s - Export G-code and save to a USB flash drive.
3	Prepare printing	- Sterile conical bioprinting nozzles, 22G - BIO X	- Make sure G-code is working and that sterile nozzles, 22G, sterile cartridges, BIO X and other equipment needed for printing are in place before proceeding to next step.
4	Prepare bioink	- GelXA SKIN bioink	- Prepare and pre-warm at least 3 mL of GelXA SKIN bioink according to the *Mixing Cells Protocol GelXA Series*. - Pre-warm the bioink by heating it in a water bath or incubator to 37°C. Note: Distribute the bioink into three syringes with at least 1 mL in each syringe. To maintain a full 24-well plate it is recommended to use 1.5 mL bioink for the reticular dermis layer.

5	Prepare cell suspension	- HDF - HEK - Nutritious solution for respectively cell type - 3 pcs 3 mL syringes with Luer lock connection	- Detach the cells according to protocol for your cells. - Prepare three cell suspensions with following quantities of cells: • HEK 10 x10^6 cells/mL bioink for the epidermis. • HDF 8 x10^6 cells/mL bioink for the papillary dermis. • HDF 4 x10^6 cells/mL bioink for the reticular dermis. Example: If you have prepared 1 mL of bioink for the epidermal layer the number of HEK needed is 10 x10^6. If you have prepared 1.5 mL of bioink for the epidermal layer the number of HEK needed is 15 x10^6. - Spin down the cell suspensions and reconstitute the cell pellet in nutritious solution so that the total volume is 100 µL per 1 mL bioink. Note: For 1 mL bioink make the total volume of cell suspension to 100 µL, for 1.5 mL bioink make the total volume of cell suspension to 150 µL. As nutritious solution complete cell culture medium usually is used.
6	Mix the GelXA SKIN bioink with the cells	- Cell suspensions - Syringes with pre-warmed GelXA SKIN bioink - 3 mL syringes with Luer lock connections - (CELLMIXER) - Female/female Luer lock adaptor - Empty, amber cartridges	At this point, mix the ten parts of bioink with the one part of cell suspension, taking care not to introduce air bubbles to the mixture. For detailed instructions see the *Mixing Cells Protocol GelX Series*. - Take the syringes with pre-warmed GelXA SKIN bioink and attach it to an empty 3 mL syringe using a female/female Luer lock adaptor. Transfer half of the bioink to each syringe. Note: To avoid introducing air into the bioink when mixing, carefully pre-fill the Luer lock adaptor with GelXA SKIN before attaching the empty syringe. - Detach the syringes and pipette the desired cell suspension into one of the GelXA SKIN containing syringes. Attach the syringes again gently mix the bioink back and forth between the syringes until the cell suspension is homogeneously incorporated. Note: Make sure to add epidermal cell suspension to the GelXA SKIN bioink prepared for the epidermis etc.

			- Transfer the GelXA SKIN bioink with cells to an empty, amber cartridge and cap the cartridge. - Repeat for all three cell suspensions. Note: If there are bubbles in the bioink, make a quick centrifugation for 1.5 min at 1600 rpm. - Place cartridge on counter for 20 min to reach room temperature. If the bioink has been pre-heated, it can instead be placed in a fridge for 3-5 min, or in the Temperature-controlled printhead at 24°C for 5 min for faster cooling. Do not attach a nozzle to the cartridge until the start of the print session. Note: If preparing bioink quantities of >2 mL of GelXA SKIN it is recommended to use a CELLMIXER to mix in the cell suspension into the bioink. For instructions see the *Mixing Cells Protocol GelX Series*.
7	Set up of the Bioprinter	- BIO X - USB with G-code	- Open the G-code on the BIO X and set the bioprinting parameters as shown below for printhead 1, 2 and 3: • Nozzle type and size: Conical tip, 410 µm (22G) • Printing pressure: 10 kPa* • Printing speed = 10 mm/s* • Printhead temperature: 24°C • Print bed temperature: 14°C - If photocuring after each construct, set the 405 nm light module to cure for 30 sec at a distance of 3 cm. If curing after printing the full plate, this is explained in detail in Step 9. *This is recommended starting parameters for the print. The pressure needed and optimal speed depends on the temperature of the surroundings and preparation procedures (amount of bioink and actual temperature of the bioink). ALWAYS test the pressure before starting the print and be prepared to adjust the speed and pressure during the print, especially for long printing sessions. Note: Since layer parameters is set in the G-code, this function is disabled on the BIO X.

8	Print-session	- The three cartridges of GelXA SKIN with cells - Sterile conical bioprinting nozzles	- Make sure the cartridges are room temperature. - Attach a 22G nozzle to each cartridge and mount cartridges with keratinocytes into printhead 1, the fibroblasts of 8 x10^6 cells/mL into printhead 2 and fibroblast of 4x 10^6 cells/mL into printhead 3. - Calibrate the printheads and start bioprinting. If printability is not as desired, adjust the pressure up/down by 1 kPa to extrude more/less material. Note: If waiting too long between extrusions the bioink can dry in the nozzle causing it to clog. If this occurs, replace with new nozzle.
9	Crosslinking	- Crosslinking Agent And/or - 405/365 nm light modules for photocuring - Thrombin - Cell culture medium	GelXA SKIN can be photocrosslinked using the 405 or 365 nm light modules and/or ionically crosslinked using the $CaCl_2$-containing Crosslinking Agent. If using both, begin with photocrosslinking. It is recommended to use both crosslinking methods which will generate the most robust constructs. Photocrosslinking only can be used for a softer construct, whilst it is not recommended to use only ionic crosslinking. Note: It is recommended to use the 405 nm light module instead of 365 nm one if possible. Overexposure might damage the cells. - Photocrosslinking: If photocuring is not preformed after each construct, cure each construct for 30 sec at a distance of 3 cm above the constructs with the 405 nm module. Note: To verify that the crosslinking is sufficient, add 37°C medium to one printed well and observe that it does not dissolve. - Ionic crosslinking: Submerge the cell-laden constructs in the crosslinking solution for 5 min. Remove crosslinking solution and rinse constructs with basal culture media once. - Thrombin: Reconstitute the thrombin in 10 mL of cell culture medium by adding 1 mL of the medium to the thrombin vial. Then transfer the 1 mL of thrombin solution back to the 9 mL of medium to receive a 10 U/mL dilution. Mix gently by pipetting up and down 2-3 times.

			- Submerge the samples in the thrombin containing medium and incubate overnight in standard culture conditions (37°C, 5% CO_2 and 95% relative humidity) or according to your application.
10	Incubation	- Cell culture medium	- The next day, switch the thrombin containing medium to regular cell culture medium and incubate in standard culture conditions or according to your application. - When air-liquid interface culture is desired; flip the constructs around so the top part (reticular dermis) becomes the base and transfer the constructs to transwell inserts. Adjust the cell culture medium so the epidermis is exposed to air. Note: It is recommended to have at least 0.3 mM calcium in the cell culture medium. For mediums containing less than 0.3 mM calcium it is required to ensure that the constructs have been properly photo cured before adding the cell culture medium to not disintegrate. Be careful with keratinocyte expansion mediums since these generally have a very low level of calcium. Time recommendations: Submerge constructs for 2-5 days before initiating air-liquid interface culture. Incubate for at least 14 days to analyse the cell viability and morphology.

Want to see our existing tissue model?
Just go to http://bioverse.co/ and discover a whole library of CAD files especially created for sharing 3D Bioprinting models.

This protocol is compatible with the use of other bioprinters. For more information, please contact: info@cellink.com

References:

N/A